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The relationship of the vascular endothelial growth factor VEGFR1 concentration Level with the bacterial infection of diabetic foot patients in Samarra city / Iraq

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ABSTRACT

The current study was conducted to estimate the relationship of vascular endothelial growth factor VEGFR1 concentration level with some bacterial infections in patients of diabetic foot. 40 samples (divided into 30 samples from diabetics patients and 10 control samples from healthy people) were collected in the form of swabs and blood samples from patients with diabetic foot who attended Samarra General Hospital and some outpatient clinics, from ages ranged between (12-68) years and of both sexes, for the period from November 2021 to the end of March 2022. a questionnaire for information about the patient was recorded.. The swabs for taking the bacterial sample included the various places of infection and were placed directly in a transport media Bacterial swabs were taken and cultured on the culture media. Microscopic diagnosis was made and biochemical tests were conducted for the isolated samples. The Analytical Profile Index System (API) was used to diagnose the types of bacterial isolates. Five ml of venous blood samples were taken from both infected and non-infected people in order to obtain serum to measure the level of VEGFR1 concentration using an ELISA device. The results of the current study showed a significant decrease of $P \leq 0.01$ in the level of vascular endothelial growth factor VEGFR1 concentration in patients with diabetic foot compared with its level in control groups. No significant differences were recorded, $P \leq 0.01$ in the level of the concentration of VEGFR1 when compared with its level in the people who have growth of bacteria (*Staph.aureus*, *Pseudomonas*, *E. coli*), while the level of the concentration of VEGFR1 significantly increased $P \leq 0.01$ among the people with diabetic foot and it was identified Bacterial growth they have with *Proteus* bacteria compared with its level in other bacteria within the subject of the current study.

Keywords: VEGFR1/ diabetic foot/ bacteria

1. Introduction

Diabetes Mellitus (DM) is a global public health threat that has increased in the last two decades [1]. In 1985, the number of diabetics reached 30 million, it rose to 285 million in 2010, and the latest global estimates issued by the International Diabetes Federation in 2019 indicate that 463 million people have diabetes, and that in 2045, the number of patients will be 700 million Approx. [2].

Patients with diabetes are exposed to multiple complications, including diabetic foot ulcers (DFUs), which are defined as a foot with ulcers or destruction and laceration of the deep tissues in the foot and is associated with peripheral neuropathy, and arterial diseases in the lower extremities of the patient with diabetes [3]. And the appearance of infections in the diabetic foot is due to a bacterial infection, and it is often multi-microbial infections [4].

Inflammation can be defined as a series of defensive tissue reactions carried out by the organism against the attacking stimuli and damage, which leads to changes in tissues and blood vessels, and is a type of non-specific immune response [5].

Vascular endothelial growth factor is a member of the VEGFR family, and is related to VEGF-A, VEGF-B, and placental growth factor. VEGFR-1 contributes to the growth of malignant tumors, but its role in the pathogenesis of angiogenesis is still poorly understood. It is considered as a method to study VEGFR-1 activation in pathological angiogenesis [6]. VEGFR-1 is a negative regulator of angiogenesis and angiogenesis during VEGFR-2 development [7].

2. OBJECTIVE

The study aims to measure the level of (VEGFR1) concentration in a unit of measurement pg/ml in diabetic foot patients and comparison with the

healthy ones, and determine the two types of bacteria (Gram positive, Gram negative) in patients suffering from diabetic foot, and then estimate the relationship between the VEGFR1 concentration and the bacterial infection that were identified within the subject ..

3. Materials and Method

The current study included 40 samples collected in the form of smears and blood samples from people with diabetic foot who attend Samarra General Hospital, as well as some outpatient medical clinics, and their ages ranged between (12-68) years, including males and females, and continued for the period from November 2021 to the end of March 2022. The information about people within the subject of the study was recorded in a special questionnaire form. The samples were divided into 30 samples for diabetic foot patients and 10 samples for healthy controls. The swabs for taking the bacterial sample included the various places of the diabetic foot and were placed directly in sterile tubes containing a transport media, and then 5 ml of venous blood samples were taken for each of the infected and non-infected, and blood serum was obtained for each person, then placed The serum is in special tubes called Epindrof tubes in order to keep them frozen at a temperature of -20 ° C for the purpose of later estimation using an ELISA device to determine the level of concentration of VEGFR1. Bacterial swabs were taken and cultured on culture media (Blood agar, Macconkey agar, Mannitol salt agar) and cultured on Nutrient agar medium for purification. Then the bacterial identification of those samples was carried out, which included microscopic identification as well as observing the staining of those isolates with gram stain and distinguishing between gram-negative bacteria and gram-positive bacteria [8]. Then the culture characteristics of the bacterial colonies such as their shape, color, texture and size were observed, and then biochemical tests were performed for the isolated samples such as mannitol and oxidase fermentation test, clotting test, catalase test and IMVIC test [9, 10]. The Analytical Profile Index System (API) was used to diagnose the types of bacterial isolates after confirming them with the aforementioned primary biochemical tests, which include (20) small tubes for biochemical tests, and the instructions of the bio Meraux company were followed and based on [11].

4. Statistical analysis

The results were statistically analyzed by applying the statistical program Minitab according to the Anova analysis of variance test and the Chi-square value test, and the arithmetic averages are compared with the Duncan polynomial test with a probability level of $P \leq 0.05$ or 0.01 .

5. Results and Discussion

- VEGFR1 concentration level

The results showed a significant decrease of $P \leq 0.01$ in the level of VEGFR1 concentration (2.45 pg/ml) among patients of diabetic foot compared with its

level in control groups (4.24 pg/ml) as shown in Table No. (1).

Table 1: Shows the concentration of VEGFR1 (pg/ml) for diabetic foot patients compared with the control.

Type of infection	Condition	VEGFR1 (pg/ml)
Diabetic foot	Patients	2.45 b
	Control	4.24 a
P-Value	0.01	

-The different letters indicate the presence of significant differences at the probability level of $P \leq 0.01$.

- The relationship between bacterial species and the immune variable VEGFR1 in patients

with diabetic foot

The results showed that there were no significant differences ($P \leq 0.01$) in the concentration of VEGFR1 (2.45, 2.39, 2.10) when comparing people with diabetic foot who were diagnosed with bacterial growth of *Staph.aureus*, *Pseudomonas*, *E.coli*, respectively, it was found that there was a significant increase ($P \leq 0.01$) in the level of VEGFR1 concentration (2.87) in people with diabetic foot who were diagnosed with bacterial growth of *Proteus* bacteria when compared with its level in people with bacterial growth *Staph.aureus*, *Pseudomonas*, *E.coli*. As shown in Table No. (2).

Table 2: shows the relationship between bacterial species and the level of concentration of the VEGFR1 in patients with diabetic foot

Type of isolation	VEGFR1 pg/ml
<i>Staph.aureus</i>	2.45 b
<i>Pseudomonas</i>	2.39 b
<i>E.coli</i>	2.10 b
<i>Proteus</i>	2.87 a
P-Value	0.01

- The different letters indicate the presence of significant differences at the probability level of $P \leq 0.01$.

- Similar letters indicate that there are no significant differences at the probability level $P \leq 0.01$

Our results agreed with the study carried out by [12], where it was found that the concentration of VEGFR1 was decreased in people with diabetic foot ulcers compared to the control. The vascular endothelial growth factor VEGFR1 is important in the process of wound healing, as well as great importance in the regeneration of damaged tissues, especially in people with diabetic foot, and the expression of this growth factor by macrophage cells and endothelial cells, and the level of its concentration It decreases in people with diabetic foot disease, and in a study conducted at Nanchang University on some animals, placental growth factor (PIGF) and vascular endothelial growth factor were injected by Adeno-Associated Virus (AAV) showing that these growth factors have a role Significant in the formation of blood vessels and

healing of wounds and injuries that occurred in the skin and tissues of the diabetic foot [13]. VEGFR1 is part of the system that re-supplies tissues with oxygen when blood circulation is insufficient, such as in hypoxia. The normal function of VEGF is to create new blood vessels during fetal development and new blood vessels after infections or diabetic foot ulcers. A major role in the formation and permeability of blood vessels, as diabetic foot ulcers lead to tissue laceration and damage to blood vessels in the foot, thus repairing this damage [12]. Monocytes and macrophage cells play an important role in the normal skin wound healing process, and the resulting defect of these types of cells leads to poor wound healing in patients with diabetes. Bone plays an important role during the inflammatory phase in wound repair by increasing its production of these cells. Dysregulated macrophage cell activity in wounds of patients with diabetes impairs the abnormal wound healing process, as these cells play important roles in

angiogenesis during wound healing. Wounds caused in diabetic foot, and this indicates a low level of VEGFR1 concentration in diabetic foot as a result of its association with the function of macrophage cells and its participation in the inflammatory process [14].

6. Conclusion

The study concluded that the level of VEGFR1 concentration was significantly decreased ($P \leq 0.01$) in patients with diabetic foot, and also decreased in those who were diagnosed with bacterial growth, as the lowest level in the concentration of VEGFR1 was in the people who were diagnosed with bacterial growth of *E. coli*, while the highest concentration was in people diagnosed with bacterial growth with *Proteus* bacteria, and this indicates the importance of VEGFR within the immune system and its importance during diabetic foot infection and recovery.

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مستوى تركيز عامل النمو البطاني الوعائي VEGFR1 وعلاقته مع بعض الانواع البكتريا التي تم عزلها من اصابات القدم السكري في مدينة سامراء / العراق

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الملخص

اجريت الدراسة الحالية لتقدير مستوى تركيز عامل النمو البطاني الوعائي VEGFR1 وعلاقته مع بعض الانواع البكتيرية لدى الاشخاص المصابين بالقدم السكري. وشملت الدراسة 40 عينة اخذت بشكل مسحات وعينات دم من مرضى المصابين بالقدم السكري المراجعين لمستشفى سامراء العام وبعض العيادات الخارجية وباعمار تراوحت بين (12-68) سنة ومن كلا الجنسين، للفترة من تشرين الثاني 2021 والى نهاية اذار 2022. وقد سجلت المعلومات الخاصة بالبحث في استمارة استبيان خاصة بمعلومات عن الشخص ضمن موضوع الدراسة. وقسمت العينات الى عينات مصابين بالقدم السكري بعدد 30 عينة وعينات السيطرة للاصحاء بعدد 10 عينات. وشملت المسحات الخاصة باخذ العينة البكتيرية مختلف اماكن الاصابة ووضعت مباشرة في انابيب معقمة وحماية على وسط ناقل (Transport media). وتم اخذ 5 مل من عينات الدم الوريدي لكل من المصابين وغير المصابين لغرض الحصول على المصل لقياس مستوى تركيز VEGFR1 باستخدام جهاز ELISA. وتم اخذ المسحات البكتيرية واجراء لها عملية الزرع على الاوساط الزرعية ومن تم اجراء التشخيص المجهرى واجراء الفحوصات الكيموحيوية للعينات المعزولة وأستخدم نظام التشخيص (API) Analytical Profile Index System لتشخيص أنواع العزلات البكتيرية. بينت نتائج الدراسة الحالية بوجود انخفاض معنوي $P \leq 0.01$ في مستوى تركيز عامل النمو البطاني الوعائي VEGFR1 لدى الاشخاص الذين المصابين بالقدم السكري مقارنة مع مستواه لدى مجاميع السيطرة. ولم تسجل فروق معنوية $P \leq 0.01$ في مستوى تركيز VEGFR1 عند المقارنة مع مستواه لدى الاشخاص الذين لديهم نمو ببكتريا (*E. coli*, *Pseudomonas*, *Staph.aureus*) في حين ارتفع مستوى تركيز VEGFR1 بشكل معنوي $P \leq 0.01$ لدى الاشخاص المصابين بالقدم السكري وتم تشخيص نمو بكتيري لديهم ببكتريا *Proteus* مقارنة مع مستواه لدى انواع البكتريا الاخرى ضمن موضوع الدراسة الحالية.